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# High Prevalence of Hepatitis G Viremia Among Kidney Transplant Patients in Thailand

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Patients receiving kidney transplants (KT) are at high risk for blood borne viral infections. To determine the prevalence of a recently discovered hepatitis G virus (HGV) in this patient group, reverse transcription-polymerase chain reaction (RT-PCR) employing primers derived from the NS5 region of the viral genome was utilized. HGV RNA was detected in 40 of 94 KT patients (43%), as compared to 3 of 69 healthy subjects (4.3%). Cocirculation of HGV and hepatitis C virus (HCV) RNA was detected in 12 patients (13%). Comparison of patients with and without HGV revealed that the former had received hemodialysis before transplantation for a significantly longer duration than the latter (28 vs. 17 months, respectively; $P < 0.05$). The amount of blood transfused and mean levels of liver enzymes, including alkaline phosphatase, alanine transaminase, and aspartate transaminase, were the same in both groups. Sequence analysis of 275-base pair DNA clones obtained from 2 patients revealed approximately 92% sequence homology to the published HGV and GB virus C sequences. These results suggested that HGV infection among Thai KT patients was high and the role of HGV in causing liver disease remains to be determined. ***J. Med. Virol. 53:162–166, 1997.***


**KEY WORDS:** HGV; GB virus C; hepatitis C virus; hepatitis B virus; hemodialysis

## INTRODUCTION

Hepatitis A, B, C, D, and E viruses do not account for all acute and chronic hepatitis. Posttransfusion hepatitis still occurs among recipients of blood units which have been screened for antibody to 2 major blood borne hepatitis viruses, hepatitis B and C viruses (HBV and HCV). Using molecular approaches, Simons et al. [1995] identified a novel hepatitis agent designated GB virus C (GBV-C) in the serum of a patient from West Africa and subsequent GBV-C strains were isolated from East Africa, Canada, and the United States. More recently, a blood-borne hepatitis virus designated hepatitis G virus (HGV) was identified in the plasma of a patient with chronic hepatitis in the United States [Linnen et al., 1996]. Analyses of nucleotide and deduced amino acid sequences revealed these 2 viruses to be different isolates of the same virus. GBV-C or HGV has a RNA genome whose genetic organization resembles those of viruses in the *Flaviviridae* family and is similar in sequence to HCV [Leary et al., 1996b]. However, its nucleotide sequence is too diverse to be classified as a HCV genotype.

The clinical significance of this new HGV is currently unclear. A high prevalence of the virus has been demonstrated in subjects with frequent parenteral exposure, including intravenous drug users, patients receiving blood transfusion, patients on hemodialysis, and patients with hemophilia [Aikawa et al., 1996, Bowden et al., 1996; Linnen et al., 1996; Masuko et al., 1996; Tsuda et al., 1996]. It is therefore likely that the HGV is transmitted primarily parenterally. Transmission of the virus by blood transfusion has recently been documented using DNA sequencing of the PCR products from donors and recipients [Schmidt et al., 1996].

At present, the only method to identify HGV infection is by reverse transcription-polymerase chain reaction using primers deduced from different regions of the viral genome such as the helicase-like NS3 [Simons et al., 1995; Leary et al., 1996a] and the NS5 [Linnen et

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al., 1996] regions. In this study, to determine the prevalence of this new virus in a Thai population, we utilized a primer pair selected from the NS5 region in a RT-PCR reaction and used primers described by Linnen et al. [1996] as nested primers. HGV infections in kidney transplant patients (a high-risk group for parenterally transmitted hepatitis) and apparently healthy subjects were investigated. Here we reported a relatively high prevalence of HGV in these populations as compared to that in patients with other parenterally transmitted HBV and HCV.

## MATERIALS AND METHODS

### Study Population

Serum samples and clinical data were collected from 94 consecutive patients who received both cadaveric and living kidney transplants at the Renal Unit, Siriraj Hospital, Bangkok, Thailand. Informed consent was obtained from all. These patients had no clinical evidence of hepatitis at the time of study. All had stable renal graft functions. Most received cyclosporin plus other immunosuppressive drugs such as prednisolone with or without azathioprine.

As a control population, serum samples were obtained from Thai military personnel who came for an annual health check-up at the Pramongkutklao Hospital, Bangkok, Thailand. Military personnel who had serum levels of alanine aminotransaminase (ALT) or aspartate transaminase (AST) exceeding the normal value (ALT > 40 U/L, AST > 37 U/L) were excluded. Sixty-nine control samples were obtained.

### Serology

Serology for antibody to hepatitis B core antigen (anti-HBc) and HCV (anti-HCV) was performed by a microparticle enzyme immunoassay using commercial kits according to the manufacturer's instructions (IMx system, Abbott Laboratories, Abbott Park, IL).

### Determination of HCV RNA by RT-PCR

RNA was extracted from 100 μl of serum using an acid guanidinium thiocyanate phenol chloroform extraction method [Chomczynski and Sacchi, 1987]. The RT-PCR and nested PCR to detect hepatitis C virus genome was performed using primers from the conserved 5′noncoding region of the HCV genome according to the method of Okamoto et al. (1990). The RNA equivalent to 10 μl of serum was used per each RT-PCR reaction.

### Determination of HGV RNA by RT-PCR

A RT-PCR was performed using oligonucleotide primers selected from the NS5 region of the HGV genome (nucleotide 6842 to 7116, GenBank number U44402) [Linnen et al., 1996]. Briefly, 2.5 μl of RNA extract (equivalent to 10 μl of serum) was mixed with 10 picomoles of anti-sense primer 7116 (5′-GAGCCACGTTGAAGACACTT-3′). The mixture (5 μl) was heated to 95°C for 5 min and cooled on ice. RT was performed in a 12.5 μl reaction containing denatured RNA-primer mixture above, 50 mM Tris, pH 8.3, 70 mM KCl, 10 mM $MgCl_2$, 10 mM DTT, 200 μM of each dNTP (Perkin Elmer, Roche, Branchburg, NJ), 20 units of RNAsin (Promega, Madison, WI), and 1.5 U of RAV-2 reverse transcriptase (Amersham, Arlington Heights, IL). The mixture was incubated at 42°C for 90 min.

The resulting cDNA was first amplified in a 50 μl reaction containing 3.5 mM $MgCl_2$, 55 mM KCl, 20 mM Tris, 75 μg/ml of gelatin, 200 μM of each dNTP, 25 picomoles of each external primer 7116 and 6842 (sense, 5′-GAATGCTGCGAGGATTCTTG-3′), and 1.25 U of AmpliTaq polymerase (Perkin Elmer, Roche, Branchburg, NJ). The mixture was denatured for 5 min at 95°C. Amplification was performed for 30 cycles as follows: denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 1 min for the first 5 cycles. For the following 25 cycles, annealing time was reduced to 1 min. The final primer extension step was at 72°C for 5 min.

For the nested step, the first round of PCR product was diluted 1:50 and amplified in a reaction mixture as described above, except that the internal primers 6904 (sense, 5′-CTCTTTGTGGTAGTAGCCGAGAGAT-3′) and 7059 (antisense, 5′-CGAATGAGTCAGAGGAC-GGGGTAT-3′) were used. The primers 6904 and 7059 are identical to primers 77F and 211R described by Linnen et al. [1996]. PCR was performed for 30 cycles as follows: denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, except that the denaturation time of the first cycle was 1.5 min and the final extension step was 5 min. The size of the amplified fragment was determined by gel electrophoresis. The expected size of the first and nested PCR products were 275 and 156 basepairs, respectively.

### Nucleotide Sequences of HGV Isolates

The products of RT-PCR amplified with primers 6842 and 7116 were cloned into pCRII vectors (Invitrogen, San Diego, CA). Nucleotide sequences were determined using Sequenase DNA sequencing kit (United States Biochemical, Cleveland, OH). DNA sequences obtained were aligned and sequence homology was calculated using Align, a sequence alignment software package (Scientific & Educational Software, State Line, PA).

### Statistical Analysis

Differences in the frequency between study groups were analyzed using the Chi-square and Fisher's exact tests. Group means were compared with the Student's t-test. *P*-values of less than 0.05 were considered of statistical significance.

## RESULTS

Forty of 94 patients (43%) were positive for HGV PCR. Prevalence of HGV RNA by other viral hepatitis markers are compared in Table I. Prevalence of HGV RNA in patients with anti-HBc positive and negative were not significantly different (41% vs. 46%, respectively). Prevalence of HGV RNA was higher in HCV RNA positive patients than that of the HCV RNA negative group (60% vs. 38%), but not statistically signifi-

TABLE I. Prevalence of HGV RNA by Other Markers of Viral Hepatitis

| Viral markers[a] | Total | HGV RNA positive |
|---|---|---|
| Anti-HBc (+) | 59 | 24 (41%) |
| Anti-HBc (−) | 35 | 16 (46%) |
| HCV RNA (+) | 20 | 12 (60%) |
| HCV RNA (−) | 74 | 28 (38%) |
| Anti-HCV (+) | 16 | 9 (56%) |
| Anti-HCV (−) | 78 | 31 (40%) |

[a](+) = positive, (−) = negative

cant ($P = 0.12$). Prevalence of HGV RNA was similar in patients with and without anti-HCV antibody (56% vs. 40%, $P = 0.34$). In the healthy control group, 3 (4.3%) and 1 (1.4%) of 69 subjects were found to be positive for HGV and HCV RNA, respectively.

Demographic, clinical, and laboratory data for patients with and without HGV infection are compared in Table II. There was no difference in age, sex, or units of blood received. Mean duration of dialysis before kidney transplantation was significantly longer in those with HGV viremia as compared to the HGV negative group (28 vs. 17 months, respectively; $P < 0.05$). Mean levels of liver enzymes including alkaline phosphatase, ALT, and AST were the same in both groups. In contrast, mean total bilirubin for patients without HGV was higher than that for patients with HGV ($P < 0.01$). However, the mean levels of both groups were in the normal range.

To confirm the HGV origin of the PCR products, a 275-base pair DNA fragment amplified with primers 6842 and 7116 was selected from 2 KT patients and cloned into a pCRII plasmid vector. DNA sequences of both clones were obtained and compared with published HGV sequences. Figure 1 illustrates the alignment of nucleotide sequences of the two clones (HGV591 and HGV857) with previously reported sequences of 2 HGV isolates [PNF2161 and R10291; Linnen et al., 1996] and GB virus C [Leary et al., 1996b]. The HGV591 and HGV857 clones shared approximately 95% sequence homology to each other and 92% to 3 other HGV isolates.

## DISCUSSION

Knowledge of the transmission, epidemiology, and clinical significance of HGV is beginning to emerge, yet whether HGV can cause fulminant hepatitis remains controversial [Yoshiba et al., 1995; Kao et al., 1996; Kuroki et al., 1996; Alter et al., 1997] and a direct association of HGV and liver pathology is still lacking. Like HBV and HCV, HGV can be transmitted parenterally [Alter et al., 1995, Schmidt et al., 1996]; therefore, patients receiving blood transfusion, hemodialysis, or organ transplants are at high risk of being infected. In the present study, we detected HGV RNA in 43% of 94 KT patients, which was slightly lower than the 55% of hemodialysis patients (n = 58) found in Indonesia [Tsuda et al., 1996]. The high prevalence of HGV infection shown in our study and the Indonesian study is markedly different from the 3.1% (n = 519) reported for hemodialysis patients in Japan [Masuko et al., 1996]. The differences may be due to geographic distribution of the virus as well as more stringent screening of blood or organ donors of known blood-borne viruses in different countries which also reduce the chance of getting infected with unknown pathogens.

HCV viremia was found in 21% (20/94) of the KT patients in our study, similar to the 25% (19/75) previously reported for Thai KT patients by Luengrojanakul and coworkers [1994]. Cocirculation of HCV and HGV RNA was found in 12 patients (13%) in this study. In contrast, Masuko et al. [1996] found both HGV and HCV RNA in only 1.3% (7/519) of Japanese patients on hemodialysis.

It should be noted that prevalence of HCV RNA (20/94, 21%) was higher than that of anti-HCV (16/94, 17%). The lower seroprevalence may be the result of immunosuppressive drugs that transplant patients were given to prevent graft rejection. Lower prevalence of anti-HCV as compared to HCV RNA in transplant patients has been reported previously [Pereira et al., 1992; Luengrojanakul et al., 1994].

Prevalence of HGV in the Thai general population was assessed using sera from healthy military personnel. HGV RNA detection rate in this group was 4.3% (6/69). This number is slightly higher than 0.5% to 1.7% previously reported for a general Western European populations [Bowden et al., 1996], and for healthy blood donors in the United States [Linnen et al., 1996], Japan [Masuko et al., 1996], and Italy [Fiordalisi et al., 1996]. HCV RNA was detected in 1 subject (1.4%) in this group, the rate similar to the 0.8% previously reported for Thai blood donors [8/961; Luengrojanakul et al., 1994].

HGV PCR primers sets found in the literature are derived from various regions of the HGV genome, for example, the 5′ noncoding region [Fukushi et al., 1996; Kuroki et al., 1996], the envelope region [Panda et al., 1996], the NS3 helicase region [Simons et al., 1995; Leary et al., 1996a; Yoshiba et al., 1995; Masuko et al., 1996; Fiordalisi et al., 1996], and the NS5 region [Linnen et al., 1996]. In our study, we selected the outer PCR primers from the NS5 region and used primers 77R and 211F described by Linnen et al. [1996] in a nested reaction. The nested PCR is likely to contribute to an increase in the sensitivity of our assay as compared to the one-step PCR procedures used by the others [Simons et al., 1995; Leary et al., 1996a; Linnen et al., 1996]. In addition, primer sequences selected to more conserved regions of the viral genome and the PCR procedures used in each laboratory may also contribute to varied sensitivity of detection, thus leading to the difference in the HGV prevalence rate.

Sequence homology between our 2 HGV clones and the corresponding regions in 2 published HGV strains (PNF2161 and R10291) and GBV-C was approximately 92%. This finding validates our PCR assay and also suggests that this region is conserved among the

TABLE II. Characteristics of 94 Kidney Transplant Recipients According to Their HGV Status

| Characteristics | HGV positive[a] (N = 40) | HGV negative[a] (N = 54) | Difference[b] |
|---|---|---|---|
| Age (year) | 40 ± 10 | 41 ± 9 | NS |
| Sex (percent male) | 45% | 63% | NS |
| Duration of dialysis (months) | 28 ± 27 | 17 ± 15 | $P<0.05$ |
| Blood transfusion (units) | 7 ± 9 | 5 ± 6 | NS |
| Liver function tests[c] | | | |
| Alkaline phosphatase (U/L) | 102 ± 56 | 93 ± 39 | NS |
| Alanine transaminase (U/L) | 31 ± 48 | 19 ± 12 | NS |
| Aspartate transaminase (U/L) | 27 ± 24 | 22 ± 9 | NS |
| Total bilirubin (μmol/L) | 12 ± 4 | 16 ± 7 | $P<0.01$ |

[a]Plus-minus values are mean ± standard deviation.
[b]NS, not significant.
[c]Normal values for serum alkaline phosphatase 39–117 U/L; alanine transaminase <40 U/L; aspartate transaminase <37 U/L; total bilirubin <17 μmol/L.

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HGV PNF2161   GAATGCTGCGAGGATTCTTGAACCGCACATTCATGTCATCATGGAGGACTGCAGTACACCCTCTCTTTGT
HGV R10291    ......................G..T..T.....................T.....................
GBV-C         ............A.....C..........C.......Y.........T.................C...
HGV 591       -......................G..A..T.....................T.................C...
HGV 857       ............................T.....................................C...

HGV PNF2161   GGTAGTAGCCGAGAGATGCCTGTATGGGGAGAAGACATCCCCCGTACTCCATCGCCAGCACTTATCTCGG
HGV R10291    .......................G.....................C.........................
GBV-C         .......................G..............A.....C...........T.............
HGV 591       ..A.................................G.......CG.A........T.....A.......
HGV 857       ..A....................G............G..........A........T..............

HGV PNF2161   TTACTGAGAGCAGCTCAGATGAGAAGACCCCGTCGGTGTCCTCCTCGCAGGAGGATACCCCGTCCTCTGA
HGV R10291    ....C.....................................................................
GBV-C         ....G.........................T.......A................C...........A..
HGV 591       ....G.............................C.....T.............C..G........A..
HGV 857       ....G.....................................C.........................G..

HGV PNF2161   CTCATTCGAGGTCATCCAAGAGTCCGAGACAGCCGAAGGGGAGGAAAGTGTCTTCAACGTGGCTC
HGV R10291    .........A..............T........T.....A..........................
GBV-C         ......T..A..............T..T..T..T...TCA........C.................
HGV 591       ......T..A..............T..T..T..T..GA.T..........................
HGV 857       ......T..A..............T..T..T..T..GA.T..-.......................
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Fig. 1. Alignment of nucleotide sequences of 5 HGV isolates. Sequences of HGV PNF2161 (nt 6842-7116), HGV R10291 (nt 6770-7044), and GBV-C (nt 6823-7097) were obtained from GenBank accession numbers U44402, U45966, and U36380, respectively [Linnen et al., 1996, and Leary et al., 1996b]. Dot denotes identical nucleotide to the HGV isolate PNF2161 shown on the first line. Dash represents space added to obtain maximum alignment.

American [Linnen et al., 1996], West African [Simons et al., 1995], and Thai strains in this report.

In summary, our study suggested that Thai kidney transplant patients are at high risk of contacting HGV infection. Lack of liver enzyme elevation as well as apparent clinical hepatitis suggested that HGV may not cause hepatitis and that if hepatitis occurred, it was mild or subclinical. Our findings are in accordance with a recent surveillance study conducted in the U.S. [Alter et al., 1997]. The U.S. study failed to detect chronic hepatitis disease in patients persistently positive for HGV RNA during 1–9 years of follow-up. Several other studies also suggest that HGV RNA can persist in the blood for years [Simons et al., 1995; Linnen et al., 1996; Masuko et al., 1996; Alter et al., 1997]. Whether HGV causes clinical hepatitis in our study population of KT patients and whether the infection is chronic will require a follow-up investigation.

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